Cell Biology

HYPOTONIC SWELLING INCREASED INTRACELLULAR CALCIUM IN ATLANTIC SALMON RED BLOOD CELLS, E.M. Helm, D.B. Light*, Department of Biology, Lake Forest College, Lake Forest, IL 60045, light@lakeforest.edu

In order for cells to survive and function properly they must maintain a normal size. When a cell is exposed to a solution that has a higher or lower concentration than its cytoplasm, water moves freely across the plasma membrane, causing the cell to change its volume. Cell swelling activates a regulatory volume decrease (RVD) mechanism, allowing cell volume to return to normal. RVD is driven by an increased permeability of the plasma membrane to specific osmolytes, which move across the membrane and effectively lower the concentration of the cytoplasm, allowing water to flow out of the cell. In *Necturus*, Ca^{2+} has been shown to be an important component of the RVD process (Light et al. 2003. *J Cell Science* 116: 101). This study examined whether intracellular Ca^{2+} levels increased during hypotonic swelling, thereby causing volume decrease in Atlantic salmon (*Salmo salor*) red blood cells. Cell volume was obtained by electronic sizing using a Coulter counter (model Z2, Beckman Coulter, Fullerton, CA). For epifluorescence studies, cells were incubated for 90 minutes with fluo-4-AM (1-10 μM), a fluorescent indicator of calcium. Intracellular Ca^{2+} levels were examined using a Nikon diaphot microscope equipped with DIC optics and epi-fluorescence (eclipse TE 2000-U). Images of cells were obtained using the computer software Metamorph (version 6.2, Universal Imaging). Hypotonic shock caused cell swelling and a concomitant increase in intracellular Ca^{2+}. Additionally, inhibition of stretch activated channels with 100 μM gadolinium reduced the increase in intracellular Ca^{2+} associated with swelling and also decreased cell volume recovery in response to hypotonic shock. This study demonstrated that cell swelling stimulates an increase in intracellular Ca^{2+}, which appears to stimulate regulated volume decrease. Future studies will address whether the source of Ca^{2+} is extracellular and/or intracellular and define a more specific role for Ca^{2+} in RVD.

E.M. Helm was supported by Lake Forest College.